Product Disposition in Periplasm Soluble Protein vs Aggregate

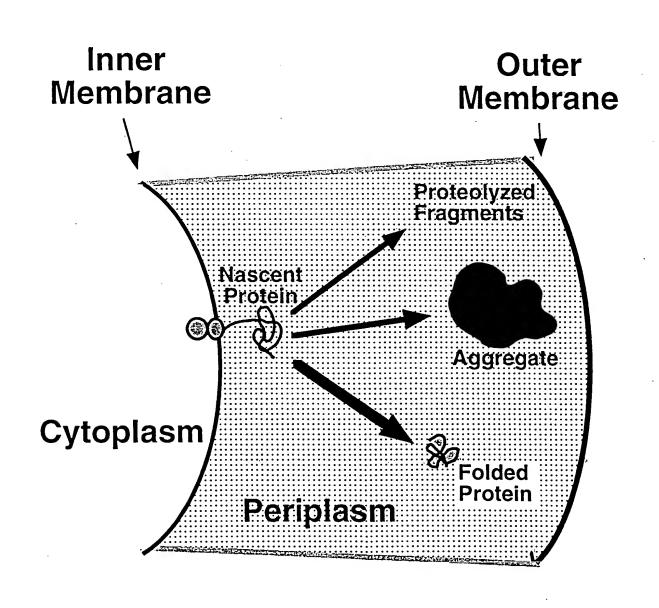


Fig. 1

Mechanical Disruption Leads to Incomplete Recovery of IGF-I Aggregates

Hart et al., Bio/Technology 12:1113 (1994)

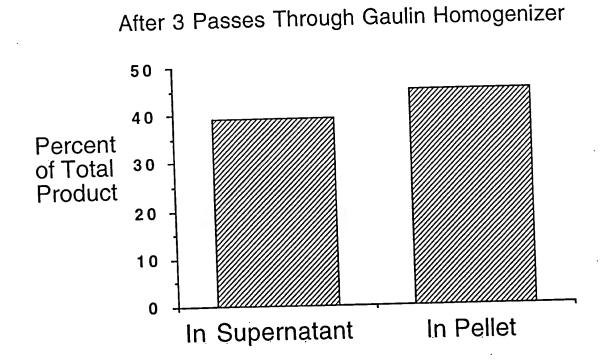
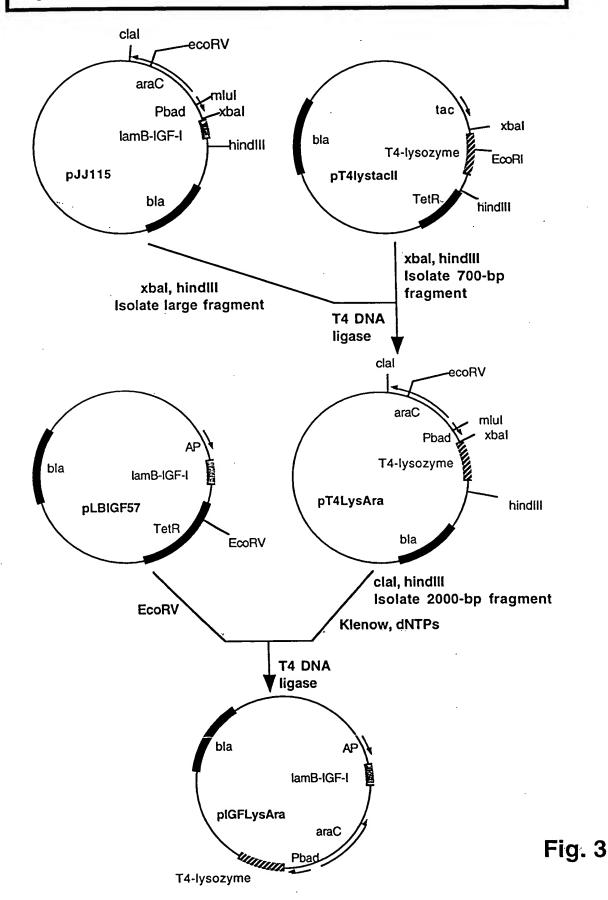


Fig. 2

plGFLysAra Plasmid Construction



Co-expression of T4-lysozyme and IGF- I by *E. coli*

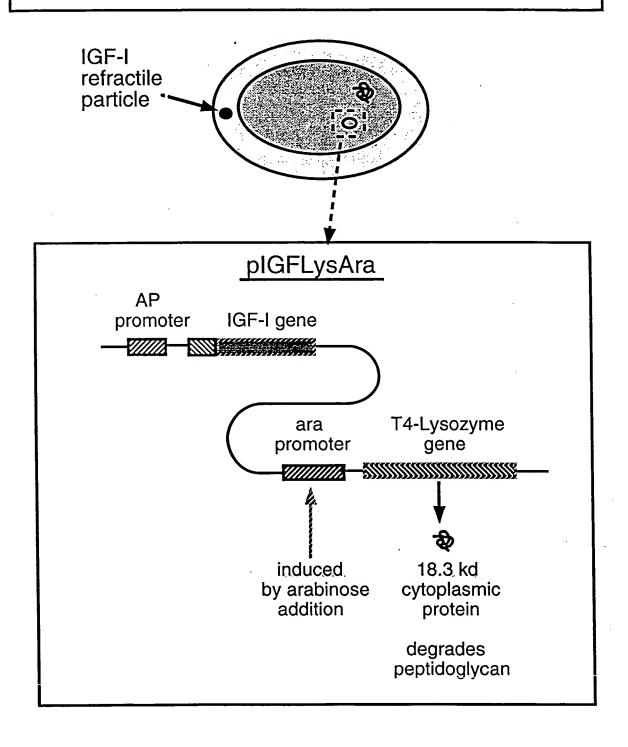


Fig. 4

rhIGF-I Fermentation Process
With Co-expression of T4-Lysozyme

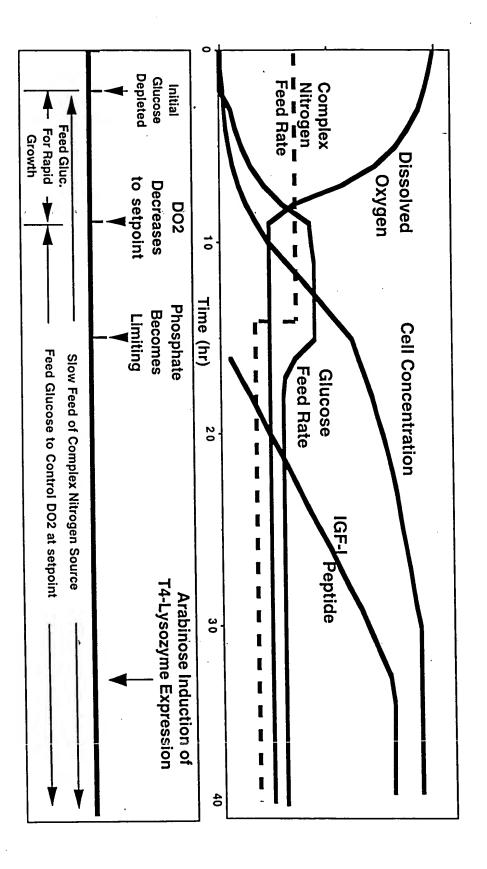
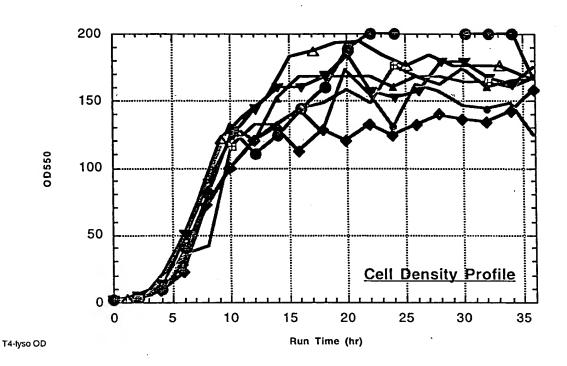


Fig. 5

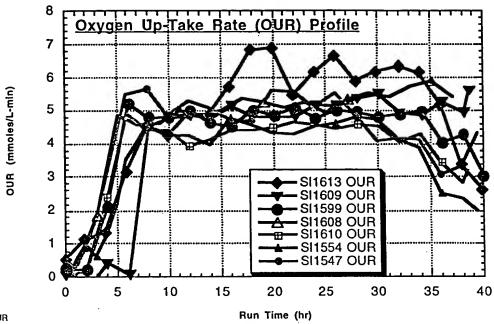
Effect of Arabinose Induction for T4-lysozyme Coexpression on Cell Density Profile



Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Effect of Arabinose Induction for T4-lysozyme Coexpression on Cellular Respiration

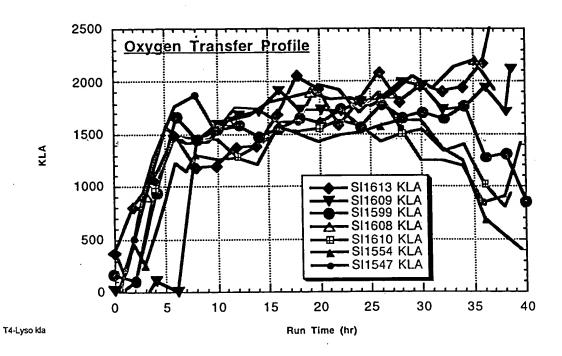


T4-lyso OUR

Run ID Key:

Run#	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Effect of Arabinose Induction for T4-lysozyme Coexpression on Oxygen Transfer during Fermentation



Run ID Key:

Run#	Production Organism_	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Effect of T4-lysozyme Co-expression on IGF-I Accumulation Arabinose Induction of pBAD Promoter

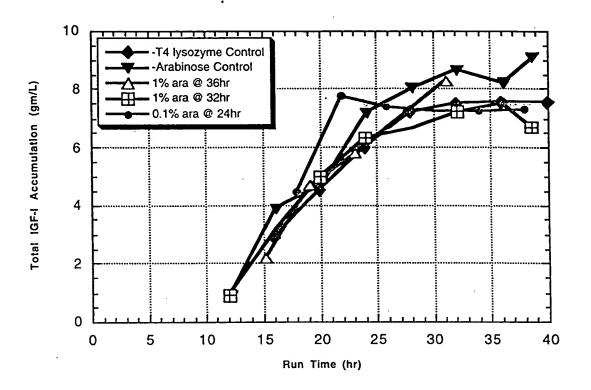
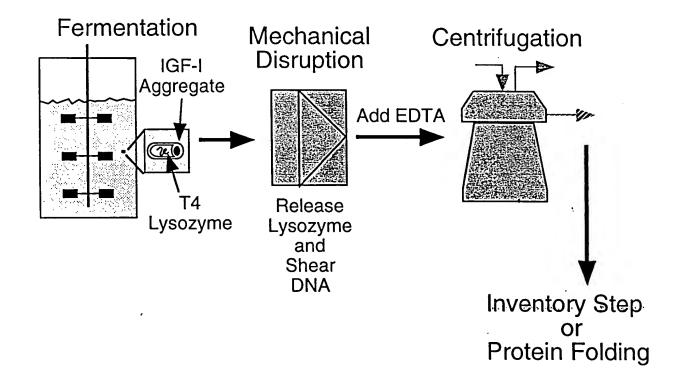


Fig. 9

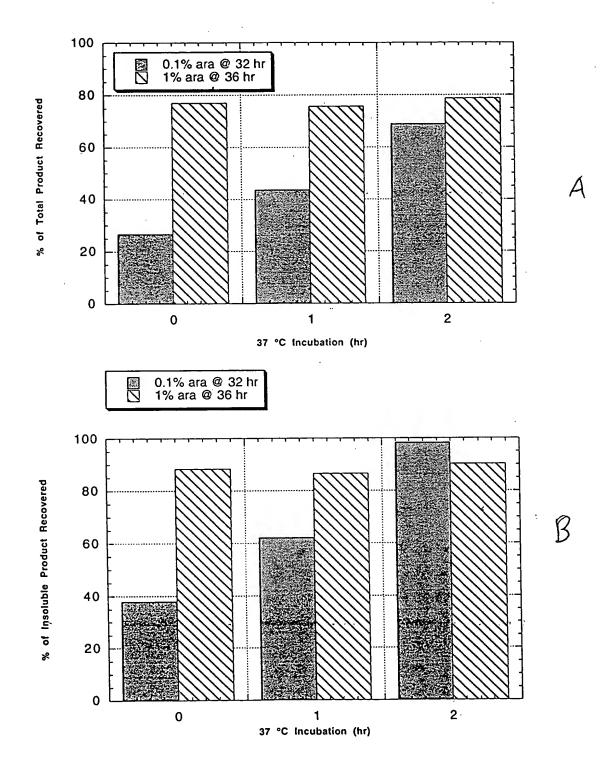
Facilitating Product Isolation Procedure With T4 Lysozyme Expression

- 1) Induce in vivo T4 Lysozyme Expression
 - * Sequested in cytoplasmic compartment

2) Isolate IGF-I Aggregates



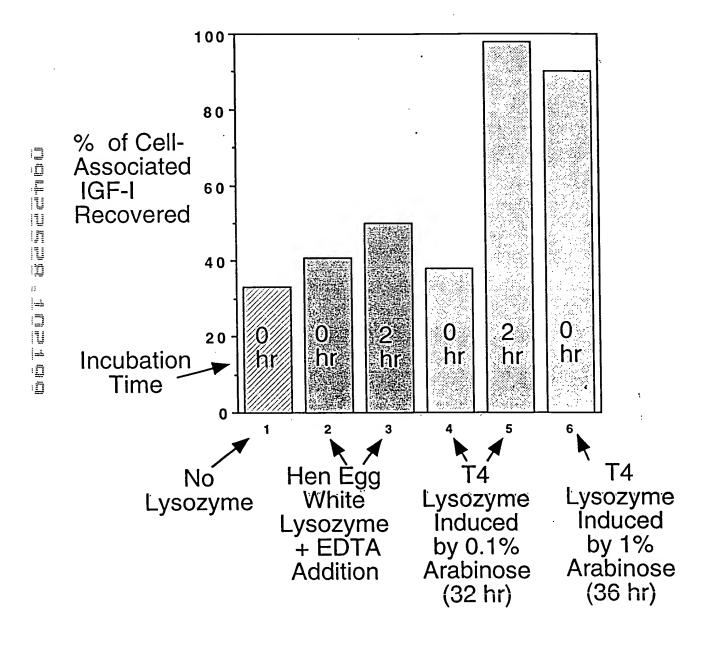
Co-Expression of T4-Lysozyme with IGF-I for Improved RP Recovery

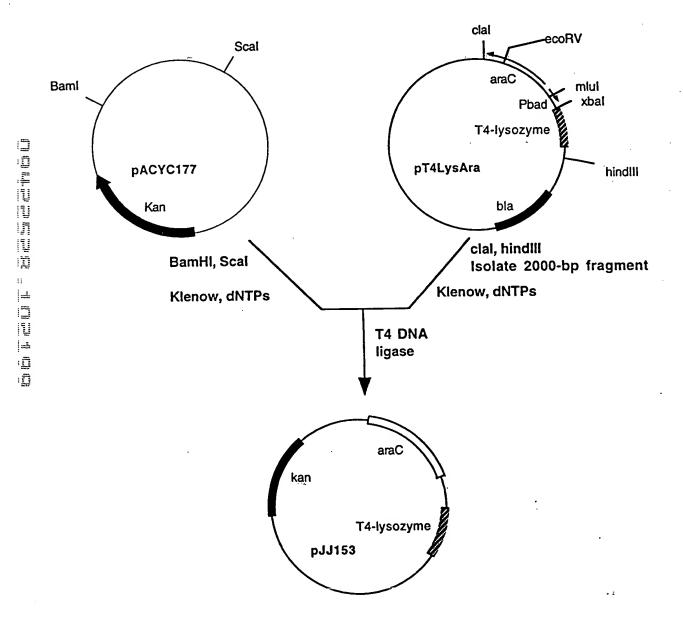


^{*} RP recovered by centrifugation at 5000 rpm X 30 min in Sorval centrifugation using GSA rotor

Facilitating Product Isolation With T4 Lysozyme Co-Expression

Results:





^Start of Tetracycline resistance

901 TGTTTGACAG CTTATCATCG ATAAGCTTTA ATGCGGTAGT TTATCACAGT TAAATTGCTA ACGCAGTCAG GCACCGTGTA TGAAATCTAA CAATGCGCTC ACAAACTGTC GAATAGTAGC TATTCGAAAT TACGCCATCA AATAGTGTCA ATTTAACGAT TGCGTCAGTC CGTGGCACAT ACTTTAGATT GTTACGCGAC

256 801 CCTTTAACTT CCAGGCTGCC TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG CTGAAGTAAG CTAATTCTCA GGAAATTGAA GGTCCGACGG ATACCGGACT ৸ z O × Ľ ഗ CACTGGTTGA CCGGGTTCGG TAGTCACTGG TGATAGGTCA CCTCCACTAC GACTTCATTC GATTAAGAGT U Ö Ľ Þ Ø Þ s S D H Y P V Ħ **X**

222 701 AGCGCTGACA CCACAGCTAC ACCCACGCAC TGTGCCTATG ACAGGATCGT GGTTGCAGGG ATGCTGCTCC GAGGCGCCGT TGTTCCCGAC TCGGCTCTTC SADT TCGCGACTGT GGTGTCGATG TGGGTGCGTG ACACGATAC TGTCCTAGCA CCAACGTCCC TACGACGAGG CTCCGCGGCAS A D T T A T P T H C A Y D R I V V A G M L L R G A V ACAAGGGCTG AGCCGAGAAG < ש U

, 601 GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC TCCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC CTACCCGCTG AAGTTACGCC CGACGTCGAT ACACTCTGGG AGGGTCACCA GTAGGTAGGC GGACACCTGT TCGGGGTGGA AGGTCACCGA CTAGGGGCTG Z a T) Z » G ဂ х Ч ۷ ۲ SWS SIR LWT SPTF O W L H U

156 501 CCCTGCATGC GGCCCCGGGG GACCGAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG GGCTTGGAGG ACGTCATGTT GGGACGTACG CCGGGGCCCC CTGGCTCATC GGCTCTAGCT GCGAGAGATA CTGCAGATGG ACCTACAGGT TCTCTTTACC CCGAACCTCC TGCAGTACAA LHA A P G D R V A EID ALY DVYL D V Q E X ი ლ E D < z L

122 401 GATGGCTGCG AGCCCTGCGG GAACGACACC TTCAACCGAG AGCCAGCCAT TGTCAGGTTC TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTC DGCE CTACCGACGC TCGGGACGCC CTTGCTGTGG AAGTTGGCTC TCGGTCGGTA ACAGTCCAAG AAGAGGGCCA AGTGTCTCCA GTCCCTCAAA CGGTAACAAG PCGGACGCC CTTGCTGTGG AAGTTGGCTC TCGGTCGGTA ACAGTCCAAG AAGAGGGCCA AGTGTCTCCA GTCCCTCAAA CGGTAACAAG PCGGACGCC \mathbb{R}

301 CGTGGTCAGT GAGCCACTGG GACGGAACAG CTATAAGGAG CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT 89 GCACCAGTCA CTCGGTGACC CTGCCTTGTC GATATTCCTC GCGATGGACA AGCACATGTC CGGACTGGTC CACAGACGCC v v s E P L G R N S YKERYLFVYR P U Q VSAV ACCIGICGAT GAIGAIGCIA U s Y ۲ ۲ U

201 ATGACATOGO COTOGTOCAG GAGGTOAGAG ACAGOOACOT GAOTGOOGTG GGGAAGOTTGO TGGACAACOT CAATCAGGAT GCACCAGACA COTATCACTA TACTGTAGCG GGACCAGGTC CTCCAGTCTC TGTCGGTGGA CTGACGGCAC CCCTTCGACG ACCTGTTGGA GTTAGTCCTA CGTGGTCTGT GGATAGTGAT DIALVQ EVRD S H L TAVGKLL U z r N O D APDT у н ү

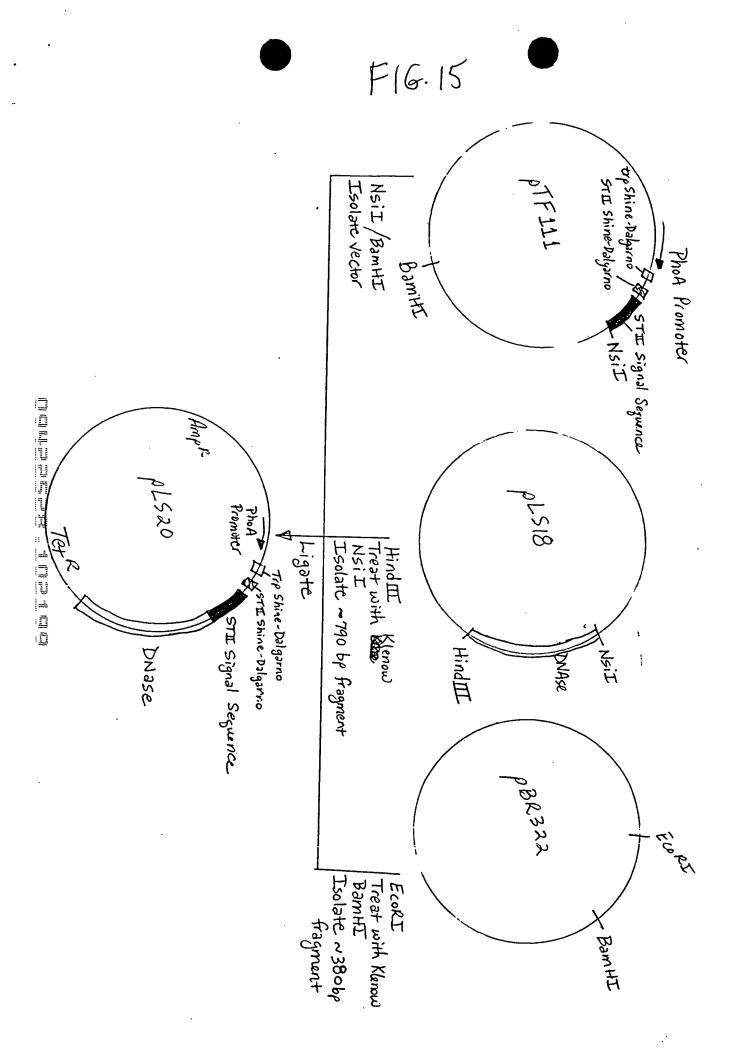
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^Start of DNase

^STII Signal Sequence

:

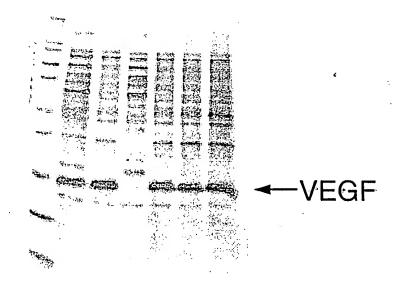
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RP Recovery Process Evaluation VEGF Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered:

MW Std Mhole Broth Pellet Supernatant M3P/LE-1hr M3P/LE-2hr M3P/LE-2hr



RP Recovery Process Evaluation DNase Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered:

